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Research letter

Effects of ethinylestradiol–cyproterone acetate vs. pioglitazone–flutamide–metformin on plasma FGF21 levels in adolescent girls with androgen excess

1. Introduction

Fibroblast growth factor 21 (FGF21) is a hormonal factor with potentially antidiabetic properties. In rodent models of obesity and type 2 diabetes (T2D), FGF21 improved glucose and lipid levels, and reduced body weight [1]. In a pilot study of obese people with T2D, short-term administration of an FGF21 analogue showed comparable effects [2]. In contrast, most clinical studies have reported high FGF21 levels in obese, insulin-resistant patients with T2D; this paradox has been attributed to a state of FGF21 resistance in obesity [3].

Hyperinsulinaemic androgen excess (HAE) is the most common cause of hirsutism and menstrual irregularity in girls and young women, and is associated with comorbidities in adulthood, including T2D [4]. Yet, only a few studies have assessed circulating FGF21 levels in women with HAE and found contradictory results; such studies included mostly obese women and were cross-sectional in design [5–7].

It has been reported that, in adolescents with HAE, insulin sensitization compared with oestrogen–progestogen contraception (OC) exerts greater beneficial effects on cardiometabolic risk markers on and off intervention. Our aim was to study whether these benefits are underscored by divergent FGF21 profiles. To this end, the present study longitudinally assessed FGF21 levels in a subset of girls with HAE.

2. Subjects and methods

2.1. Study population and design

The study population consisted of 25 non-obese adolescent girls with HAE [mean age: 16 years; body mass index (BMI): 23 kg/m^2] who were included in a randomized, open-label study to compare the effects of OC with ethinylestradiol–cyproterone acetate [EE–CA; 35 μg of EE plus 2 mg of CA for 21 days, followed by placebo for 7 days; Bayer Schering Pharma AG, Berlin, Germany] with the effects of a low-dose combination of pioglitazone 7.5 mg/day, flutamide 62.5 mg/day and metformin 850 mg/day (PioFluMet). The study was registered as ISRCTN45546616, and lasted for 24 months (18 months of treatment followed by 6 months of no treatment) [4,7]. The present report includes only those girls with complete longitudinal data whose serum samples remained sufficiently abundant to measure FGF21 at each study time point (~74% of the initial study population: $n = 14$ in the EE–CA subgroup; $n = 11$ in the PioFluMet subgroup).

2.2. Assessments

Clinical and endocrine–metabolic variables, body composition and abdominal fat partitioning were assessed as described elsewhere [4,8]. Circulating FGF21 levels were determined using a specific non-cross-reactive enzyme-linked immunosorbent assay (ELISA; BioVendor Laboratory Medicine, Brno, Czech Republic); the detection limit was 4.8 pg/mL, and the intra- and interassay coefficients of variation were 3.5% and 3.7%, respectively.

2.3. Statistical analyses

These were performed with SPSS version 12.0 software (SPSS, Chicago, IL, USA). Results are expressed as means ± SEM. Results with a non-Gaussian distribution were log-transformed before analysis. Comparisons within and between groups at each time point were performed using a general linear model. To assess the effects of treatment and time simultaneously on FGF21 levels, two-way analysis of variance (ANOVA) was performed. $P < 0.05$ was considered statistically significant.

3. Results

Both treatments had comparable effects on measures of androgen excess, but they had divergent effects on cardiometabolic risk markers and body composition: PioFluMet administration was followed by more favourable on-treatment and post-treatment outcomes, as reported (see supplementary Table for differences in selected variables) [8].

FGF21 levels in HAE girls were comparable to those in age- and BMI-matched controls at baseline (Fig. 1, Table S1). After 18 months of treatment, FGF21 concentrations increased only in the EE–CA group, and remained elevated even after therapy discontinuation ($P = 0.02$ between subgroups at both time points; Fig. 1). Treatment explained 11% of FGF21 variability ($P = 0.04$), while time explained only 2.4% ($P = 0.56$).

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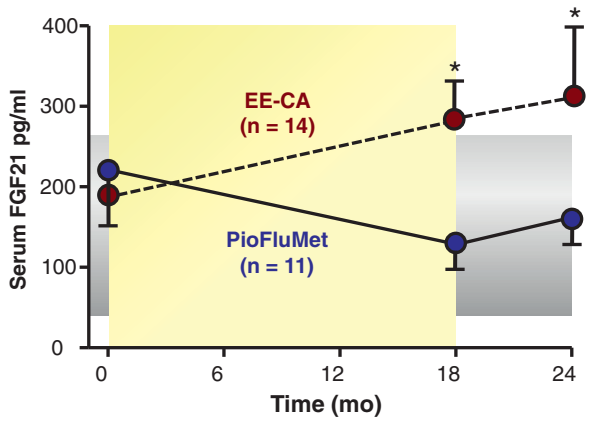

Fig. 1. Longitudinal fibroblast growth factor 21 (FGF21) concentrations (means ± SEM) in adolescent girls with hyperinsulinaemic androgen excess who were randomized to receive either an oral contraceptive [ethinylestradiol–cyproterone acetate (EE–CA); $n = 14$] or a low-dose insulin-sensitizing treatment (PioFluMet; $n = 11$), comprising pioglitazone (7.5 mg/day), flutamide (62.5 mg/day) and metformin (850 mg/day) for 18 months, with no treatment between 18–24 months. The upper and lower limits of the grey zone correspond to a Z score of +1 and –1, respectively, in healthy control girls (age: 16.4 ± 0.4 years; BMI: 22.1 ± 0.2 kg/m^2; $n = 15$). FGF21 increased significantly in the EE–CA group at 18 and 24 months. $^*P = 0.02$ between subgroups at 18 and 24 months (by two-sided t-test).

Initially, FGF21 correlated inversely with BMI and fat distribution in all patients (Table 1). At 18 and/or 24 months of treatment, FGF21 was positively associated with measures of insulin resistance, triglycerides and intrahepatic fat, and negatively associated with high-density lipoprotein (HDL) cholesterol and high-molecular-weight adiponectin (HMW-adip) in the EE–CA subgroup. In the PioFluMet subgroup, FGF21 showed an inverse relationship with BMI and intrahepatic fat, and was positively correlated with HMW-adip (Table 1).

Also, in the PioFluMet girls, changes in FGF21 levels between 0 and 18 months were negatively correlated with changes in insulin resistance measures and intrahepatic fat, and positively correlated with changes in HMW-adip levels. In contrast, in the EE–CA subgroup, FGF21 changes correlated positively with glucose and intrahepatic fat changes, and negatively with BMI and low-density lipoprotein (LDL) cholesterol changes. These correlations remained significant only for measures of insulin resistance (PioFluMet subgroup) and glucose (EE–CA subgroup) on analyses performed between 0 and 24 months (Table 1).

4. Discussion

To our knowledge, this is the first longitudinal study comparing the effects of OC vs. insulin sensitization on circulating FGF21 concentrations in non-obese adolescents with HAE. Our present findings reveal that OC administration is followed by augmented FGF21 levels that persisted even after therapy withdrawal.

FGF21 levels were within normal limits in all of our study participants at baseline and showed an inverse correlation with measures of adiposity. Thus, in non-obese glucose-tolerant individuals, FGF21 may act as a metabolic modulator, as reported in experimental studies [1]. In contrast, in overweight and obese subjects with metabolic dysfunction and/or diabetes, high endogenous FGF21 concentrations fail to exert such benefits, leading to a state of FGF21 resistance as a result of reduced expression of beta-Klotho co-receptors in metabolically key organs, including adipose tissue [9,10]. These effects appear to be independent of the presence of HAE [7].

After 18 months of treatment, FGF21 concentrations increased only in the EE–CA subgroup, and were associated with measures of insulin resistance, lipids, HMW-adip and intrahepatic fat on and/or off therapy, but not with androgen levels, which is in agreement with previous reports [7]. The increase in FGF21 persisted off treatment together with more insulin resistance and a less favourable cardiometabolic profile [4,8]. This is in line with prospective studies showing that FGF21 independently predicts progression to the metabolic syndrome in patients with metabolic dysfunction, such as those with obesity and impaired glucose homoeostasis [9–11].

Our findings have also revealed that FGF21 is a sensitive marker that increases in response to any drift in either glucose or lipid homeostasis in non-obese girls. The increase in FGF21 levels following EE–CA treatment could be a mechanism to counteract the potentially negative effects of EE–CA on intrahepatic fat and metabolic markers. Interestingly, FGF21 concentrations remained unchanged in the PioFluMet subgroup whether on or off treatment and in parallel with sustained improvements in cardiometabolic markers.

The effects of pioglitazone and metformin on circulating FGF21 levels remain controversial, although there is agreement that both agents induce FGF21 expression in cultures of rat and human hepatocytes [12,13]. The effects of metformin appear to be mediated through AMPK activation [12], whereas those of pioglitazone might be exerted through peroxisome proliferator-activated receptor gamma (PPARγ) stimulation [13]. However, pioglitazone at the low doses used in our present study is an inhibitor of cyclin-dependent kinase 5-mediated phosphorylation of PPARγ rather than a PPARγ activator [4].

The few studies performed so far in hyperandrogenic patients were cross-sectional and included mostly obese women – not adolescents – and used less restrictive inclusion criteria [5–7]; thus, the results may not be comparable.

Limitations of the present study include the relatively short follow-up period off therapy, the potential non-applicability of its conclusions to glucose-intolerant, diabetic and/or obese girls with HAE, and the fact that only the effects of EE–CA were assessed, which means the impact of other OC combinations remain to be elucidated. The strengths include its longitudinal design, the rather homogeneous study population and the assessment of two interventions with divergent effects on cardiometabolic health.

In conclusion, this report for the first time investigated FGF21 circulating levels in non-obese girls with HAE. The longitudinal design of the study allowed detection of the divergent effects of OC and insulin sensitization on serum FGF21 concentrations, and revealed that higher FGF21 levels in young girls with HAE are linked to poorer endocrine–metabolic status.

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Table 1
Correlations between FGF21 and clinical, endocrine-metabolic and imaging parameters initially and during interventions with either ethinylestradiol-cyproterone acetate (EE-CA) or low-dose pioglitazone + lutamide + metformin (PioFluMet) in girls with hyperinsulinaemic androgen excess.

	Baseline ($n = 25$)		18 months				Δ 0–18 months			
			PioFluMet ($n = 11$)		EE–CA ($n = 14$)		PioFluMet ($n = 11$)		EE–CA ($n = 14$)	
	r	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*
Body mass index	–0.470	0.019	–0.786	0.007	0.249	0.390	0.194	0.568	–0.565	0.044
Glucose	–0.158	0.449	–0.592	0.055	0.029	0.921	–0.851	0.015	0.573	0.032
Insulin	–0.130	0.540	–0.356	0.283	0.397	0.160	–0.708	0.049	–0.153	0.619
HOMA-IR	–0.360	0.075	–0.419	0.199	0.378	0.182	0.301	0.431	–0.075	0.797
AT-IR	–0.214	0.315	–0.842	0.002	0.655	0.015	–0.691	0.039	–0.532	0.075
HDL cholesterol	–0.020	0.924	0.385	0.242	–0.712	0.006	–0.208	0.564	–0.300	0.297
LDL cholesterol	0.217	0.297	0.106	0.757	–0.104	0.725	0.487	0.154	–0.563	0.034
Triglycerides	0.058	0.781	–0.067	0.845	0.548	0.04	–0.156	0.666	0.020	0.948
HMW-adip	0.042	0.844	0.701	0.024	–0.254	0.380	0.741	0.022	–0.239	0.454
Abdominal fat[a]	–0.682	0.0003	–0.338	0.308	0.134	0.649	–0.803	0.009	–0.223	0.464
Subcutaneous fat[b]	–0.431	0.036	–0.456	0.159	0.077	0.794	0.066	0.847	–0.166	0.571
Visceral fat[b]	–0.603	0.001	–0.615	0.058	0.169	0.563	–0.286	0.395	–0.345	0.228
Intrahepatic fat[b]	–0.046	0.829	–0.244	0469	0.694	0.008	–0.711	0.032	0.613	0.034

	24 months				Δ 0–24 months			
	PioFluMet ($n = 11$)		EE–CA ($n = 14$)		PioFluMet ($n = 11$)		EE–CA ($n = 14$)	
	r	*P*	*r*	*P*	*r*	*P*	*r*	*P*
Body mass index	–0.628	0.038	0.093	0.749	–0.502	0.116	–0.204	0.484
Glucose	0.032	0.925	–0.228	0.434	–0.047	0.890	0.601	0.029
Insulin	0.446	0.196	0.627	0.022	–0.252	0.454	–0.276	0.340
HOMA-IR	0.471	0.169	0.587	0.035	–0.342	0.303	–0.232	0.426
AT-IR	0.114	0.739	0.125	0.671	–0.685	0.042	–0.181	0.537
HDL cholesterol	0.470	0.145	–0.582	0.037	0.381	0.247	–0.414	0.141
LDL cholesterol	0.219	0.517	–0.064	0.827	–0.194	0.591	0.211	0.468
Triglycerides	–0.171	0.615	0.119	0.685	–0.109	0.765	0.221	0.469
HMW-adip	0.347	0.295	–0.660	0.038	0.523	0.149	–0.373	0.209
Abdominal fat[a]	–0.310	0.353	0.143	0.640	–0.200	0.555	–0.210	0.491
Subcutaneous fat[b]	–0.265	0.430	–0.252	0.455	0.296	0.376	0.153	0.601
Visceral fat[b]	–0.321	0.336	0.277	0.360	–0.221	0.513	–0.189	0.535
Intrahepatic fat[b]	–0.661	0.027	0.222	0.445	0.145	0.671	–0.218	0.453

HOMA-IR: homoeostasis model assessment for insulin resistance; AT-IR: adipose tissue insulin-resistance index (serum free fatty acids x fasting insulin; Lomonaco et al. [14]); HDL/LDL: high-density/low-density lipoprotein; HMW-adip: high-molecular-weight adiponectin.
[a] By absorptiometry.
[b] By magnetic resonance imaging (MRI).

Disclosure of interest

The authors declare that they have no competing interest.

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Appendix A. Supplementary data

Supplementary data (Table S1) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.diabet.2015.10.004.

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